Malays. Appl. Biol. (2014) 43(1): 65–71

SYNERGISTIC EFFECTS OF GSK3 AND p38 MAPK INHIBITORS ON GROWTH OF *Plasmodium falciparum EX VIVO*

MARHALIM, L., EMBI, N. and SIDEK, H.M.*

School of Biosciences and Biotechnology,
Faculty of Science and Technology,
Universiti Kebangsaan Malaysia, Bangi, 43600, Selangor
**Email: hasidah@ukm.edu.my*

ABSTRACT

Pharmacological inhibitors of glycogen synthase kinase 3 (GSK3) and p38 mitogen-activated protein kinase (p38 MAPK), two kinases commonly associated with signaling within cells, have been shown to suppress *in vivo* or *ex vivo* growth of plasmodial sp. Here we evaluated antiplasmodial activities *ex vivo* of four inhibitors against GSK3 [lithium chloride (LiCl), kenpaullone, (2'Z, 3'E)-6-bromoindirubin-3'-oxime (BIO) and SB216763]; and two against p38 MAPK (RWJ67657 and SB202190) individually and in combination preparatory to understanding the role of protein kinases in plasmodial development. The order of decreasing growth-suppressing potencies of the GSK3 inhibitors tested against *Plasmodium falciparum* 3D7 cultured *ex vivo* in erythrocytes was BIO (IC_{50}=3.13 μM) > kenpaullone (IC_{50} = 18.3 μM) > SB216763 (IC_{50}= 27.12 μM) > LiCl (IC_{50}= 25 468 μM). The p38 MAPK inhibitor, RWJ67657, displayed an IC_{50} of 7.52 μM against 3D7. SB202190 was less effective at inhibiting 3D7 displaying an IC_{50} of 14.80 μM. When tested in combination, marked synergism was observed for combination of BIO or SB216763 with RWJ67657. In conclusion, GSK3 and MAPK inhibitors showed potent antiplasmodial activities. Synergistic effects observed between BIO or SB216763 and RWJ67657 in inhibiting plasmodial growth may implicate interaction between MAPK and GSK3 pathways and warrant further investigation.

Key words: GSK3, MAPK, inhibitors, *Plasmodium falciparum*, malaria

INTRODUCTION

Malaria remains a major human disease today with 3300 million people at risk of infection in 2010 despite serious strategies to eradicate the disease. Approximately 90% of malaria-related deaths in sub-Saharan Africa occur essentially among children below the age of five (WHO, 2012). *Plasmodium falciparum* is one of four *Plasmodium* species affecting humans and the most deadly. Rapid development of parasite resistance towards available antimalarial drugs (chloroquine, mefloquine, quinine, pyrimethamine, artemisinin) presents a significant challenge to prevention and elimination efforts for the disease (Winstanley, 2000). This has escalated research for novel drug targets of which protein kinases have attracted wide attention (Doerig 2004).

Protein kinases represent attractive candidates as drug targets in diseases with dysfunctional phosphorylation activities. Gleevec, a drug introduced for the treatment of leukemia, bone marrow disorders and skin cancer was the first protein kinase inhibitor to have been commercialized (Cohen 2002). Because of their essential roles in crucial cellular functions in eukaryotes, protein kinases are also attractive potential targets for drugs against eukaryotic infectious agents such as plasmodium and trypanosomatids. In malarial infections, inhibitors of glycogen synthase kinase 3 (GSK3) and mitogen-activated protein kinase (MAPK), two protein kinases with essential roles in the plasmodial life cycle, have been reported to individually suppress development of plasmodial sp. (Drocheau *et al.,* 2004, Brumlik *et al.,* 2011).

GSK3 is a serine/threonine kinase first discovered in 1980 for its role in glycogen metabolism (Embi *et al.,* 1980). More than forty substrates phosphorylated by GSK3 have since been identified thus illustrating the capability of GSK3 to affect diverse cellular processes from glycogen and protein synthesis, to the regulation of transcription factors, embryonic development, cell proliferation and adhesion, tumorigenesis, apoptosis and neuronal death (Doble & Woodgett, 2003).

* To whom correspondence should be addressed.

The mitogen-activated protein kinase (MAPK) pathway, a three-tiered cascade of phosphorylation-dephosphorylation found in all eukaryotes, is a vital mediator of a number of cellular fates including growth, proliferation, survival, apoptosis, inflammation and cell death (Chen *et al.,* 2001, Hommes *et al.,* 2003). There are three major distinctly-regulated groups of MAPK cascades known in humans; extracellular regulated protein kinase ½ (ERK½), c-Jun NH2 terminal kinase (JNK) and p38 MAPK. MAPK cascades play a central role in progression of Alzheimer's disease, cancer development, neuro-inflammatory responses as well as neuronal death in Parkinson's disease (Kim & Choi 2010).

Knowledge on molecular mechanisms involved in combination therapies may provide clues in understanding pathways with specific mechanisms of action. Both GSK3 (Meijer *et al.,* 2004) and MAPK (Lawrence *et al.,* 2008), which are known to mediate normal signaling in multiple cellular processes, are implicated in the pathogenesis of malaria. Interestingly, it has been reported that direct phosphorylation by p38 MAPK may inactivate GSK3β activity (Thornton *et al.,* 2008). Here we set out to investigate if pathways involving GSK3 and MAPK may interact to cause suppression of plasmodial growth.

MATERIALS AND METHODS

P. falciparum cultivation and maintenance

3D7, a chloroquine-sensitive *P. falciparum* strain, obtained from the Malaria Research and Reference Reagent Resource Centre (MR4, Manassas, Virginia) was grown and maintained in culture at 1% hematocrit of purified O+ human erythrocytes in RPMI 1640 media supplemented with 0.5% Albumax II (GIBCO, Life Technologies, USA), 25 mM HEPES, 100 μM hypoxanthine, 12.5 μg/mL gentamicin and 1.77 mM sodium bicarbonate at 37°C, 5% CO_2 (Radfar *et al.,* 2009). The parasitemia level was evaluated by microscopic inspection of Field-stained thin smears.

Drug and inhibitors

Chloroquine diphosphate was purchased from Sigma Chemical Company (USA). The GSK3 inhibitor, lithium chloride was purchased from Fluka (USA), kenpaullone and SB216763 were obtained from Merck (Germany), and (2'Z,3'E)-6-bromoindirubin-3'-oxime (BIO) from Sigma Chemical Company (USA). The p38 MAPK inhibitors RWJ67657 and SB202190 were purchased from Tocris Bioscience (USA).

Determination of inhibitor susceptibility *in vitro*

Susceptibility of the 3D7 strain of *P. falciparum* to various inhibitors was measured using WHO microtest and parasite lactate dehydrogenase (pLDH) assays. Parasite LDH assays were performed according to Makler *et al.* (1993) in flat-bottomed 96-well microtiter plates. Unparasitized O^+ erythrocytes without inhibitors were used as blanks for the assay; and parasitized erythrocytes without inhibitors served as controls for the assay. Parasites were plated in the 90% ring phase at 1% hematocrit and 2% parasitemia in 100 μL of inhibitors at defined concentrations. Plates were placed in the CO_2 incubator and incubated for 48 hours. When incubation was complete, the plates were subjected to three 20 minute freeze-thaw cycles. Thereafter, 100 μL Malstat reagent (0.2% (v/v) Triton X-100, 0.02 g/mL L-lactate, 6.6 mg/mL Tris buffer, 0.11 mg/mL 3-acetylpyridine adenine dinucleotide (APAD), pH 9 and 25 μL NBT/PES solution (1.6 mg/mL nitro blue tetrazolium, 0.08 mg/mL phenazine ethosulfate) were added to each well of fresh 96-well microtiter plates. Culture (15 μL) was taken from each well and added to the corresponding well of the Malstat plates. Color development in the plates were monitored at 620 nm using a microplate reader (Fluostar Optima) after an hour of incubation at room temperature in the dark. Data were analyzed using GraphPad Prism 5 (GraphPad Software, Inc., San Diego, CA) by non-linear regression to yield 50% inhibitory concentrations (IC_{50}).

Stage-specific developmental inhibition

Highly synchronized parasite ring stage cultures obtained by D-sorbitol treatment (Radfar *et al.,* 2009) at 2% parasitemia and 5% hematocrit, were exposed to GSK3 or p38 MAPK inhibitors using concentrations required to achieve 90% growth inhibition as obtained earlier from the inhibitor susceptibility (pLDH) assay (Skinner *et al.,* 1997). Drug or inhibitor exposure studies were performed at 0 hour (ring stage), 28 hour (trophozoite stage), 42 hour (schizont) and 54 hour (ring). Purity of individual stages was confirmed to be greater than 95% by light microscopic examination of field-stained thin smears of the cultures (Dobson *et al.,* 2001).

Ex vivo inhibitor combination bioassay

Method described by Canfield *et al.* (1995) was adopted. Briefly, every fixed ratio of predetermined concentrations needed to inhibit parasite growth by 50% (IC_{50}) was used to determine the interaction of two inhibitors. Initial concentrations 20-50 times the estimated IC_{50} values combined in nine

combination ratios of 90:10 to 10:90 (inhibitor A: inhibitor B) of three GSK3 inhibitors and RWJ67657 were tested against *P. falciparum* 3D7 (Kebeneil *et al.,* 2004). Highest concentrations of each inhibitor were prepared to allow the IC_{50} of the individual inhibitor to fall around the midpoint in a two-fold serial dilution. Combined inhibitors were dispensed into the 96-well microtiter plates and subsequent procedures were followed as described in the determination of inhibitor susceptibility *in vitro* described above.

The degree of synergy was evaluated according to the method of Berenbaum *et al.* (1978). Fraction inhibition concentration (sum FIC) was calculated using the formula:

$$Ac/Ae + Bc/Be = K \text{ (sum FIC)}$$

where Ac and Bc are the equally effective concentrations (IC_{50}) when used in combination, and Ae and Be are the equally effective concentrations when used alone. FICs < 1 indicates synergism, sum FICs ≥ 1and < 2 indicates additive interaction and sum FIC ≥ 1 indicates antagonism (Gupta *et al.,* 2002).

RESULTS

GSK3 and p38 MAPK inhibitors individually suppressed *P. falciparum* growth

We first evaluated effects of a panel of GSK3 and p38 MAPK inhibitors (lithium chloride, kenpaullone, BIO, SB216763, RWJ67657 and SB202190) individually on growth of *P. falciparum* 3D7 in culture. The 50% inhibition concentrations for each inhibitor tested are shown in Table 1. Of the GSK3 inhibitors examined, BIO exhibited the most potent GSK3 inhibitory effect on growth of the chloroquine-sensitive 3D7 parasite strain with an IC_{50} of 3.13 μM (Table 1). The order of decreasing inhibitory activity for the GSK3 inhibitors tested is BIO > kenpaullone > SB216763 > lithium chloride. As for p38 MAPK inhibitors tested, RWJ67657 showed more effective inhibition compared to SB202190 displaying IC_{50} values of 7.52 μM and 14.80 μM respectively.

GSK3 and p38 MAPK inhibitors arrested plasmodial ring-trophozoite transition stage

Next we examined the 3D7 parasite developmental stage affected by the GSK3 and p38 MAPK inhibitors. Comparisons between Giemsa-

Table 1. *In vitro* anti-plasmodial activity

Drug/Inhibitor		Antiplasmodial activity (*P. falciparum* 3D7), IC_{50} (μM)	IC_{90} (μM)
GSK3	Lithium Chloride	25 468 ± 1.109	213 796
	Kenpaullone	18.30 ± 1.202	169.8
	BIO	3.13 ± 1.249	11.22
	SB216763	27.12 ± 0.0013	93.3
p38 MAPK	RWJ 67657	7.52 ± 1.285	66
	SB202190	14.8 ± 1.286	100
Control	Chloroquine	0.0084 ± 0.0011	0.063

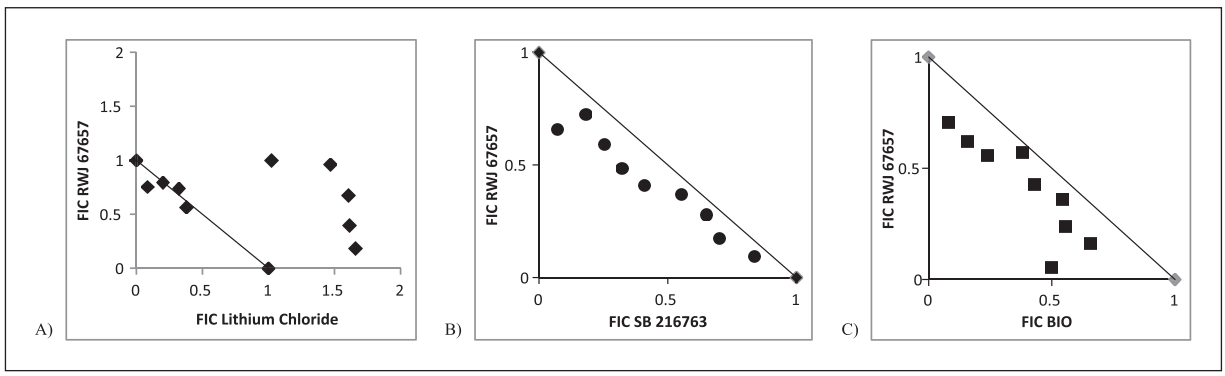

Fig. 1. Normalized isobolograms showing the interaction between GSK3 inhibitors; lithium chloride, SB216763 and BIO with RWJ67657 against *P. falciparum* 3D7 strain.

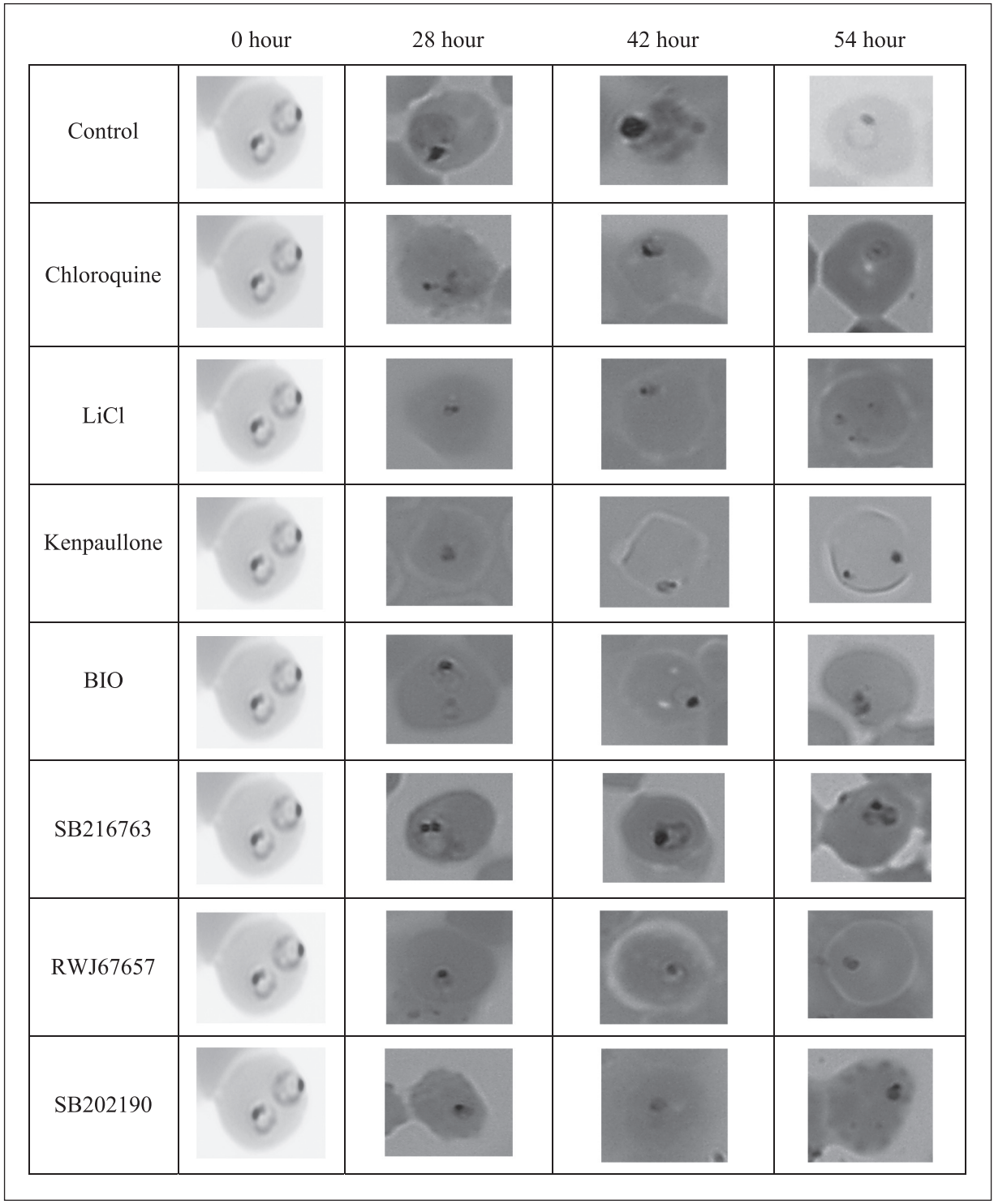

Fig. 2. Morphological changes in parasites observed after incubation with GSK3 and p38 MAPK inhibitors. Highly synchronous *P. falciparum* 3D7 cultures were exposed to Lithium chloride (213 mM), Kenpaullone (170 μM), BIO (11 μM), SB216763 (93 μM), RWJ67657 (66 μM), SB202190 (100 μM) and Chloroquine (0.063 μM).

stained thin blood smears prepared from the parasite cultures treated with the inhibitors against non-treated controls revealed that control non-treated cultures completed their intra-erythrocytic development within the asexual cycle. This was deduced from the presence of ring, trophozoite, schizont, and ring morphologies as observed under the microscope in cultures obtained at the 0, 28, 42 and 54 hour time-points respectively (Figure 2). In contrast, only ring stage parasites were observed in cultures treated with inhibitors within the same time-points. We also observed that developmental retardation yielded trophozoites that were diminished in size with lesser pigment colour

Table 2. Sum FICs for the interactions of GSK3 inhibitors – RWJ67657 against 3D7 *P. falciparum*

Inhibitor combination	Fixed combination ratio (Mean of FIC_{50})								
	90:10	80:20	70:30	60:40	50:50	40:60	30:70	20:80	10:90
Lithium chloride + RWJ67657	1.841	2.01	2.282	2.425	2.021	0.943	1.059	0.993	0.839
SB216763 + RWJ67657	0.929	0.876	0.929	0.923	0.819	0.806	0.843	0.908	0.732
BIO + RWJ67657	0.554	0.822	0.790	0.904	0.857	0.953	0.798	0.777	0.788

starting at 28 hours of culture in the presence of all tested inhibitors (Figure 2). The latter observation is indicative of successful inhibition of parasite developmental stage by the GSK3 and p38 MAPK inhibitors. The results obtained strongly suggest that GSK3 and p38 MAPK inhibitors caused stage-specific growth inhibition specifically suppressing conversion of ring to trophozoite morphologies.

More profound inhibition on growth of *P. falciparum* was observed with combination of inhibitors

The antiplasmodial activities of GSK3 inhibitors (lithium chloride, SB216763 and BIO) in combination with RWJ67657 were investigated against 3D7 *P. falciparum* culture. The mean sums of the fractional inhibitory concentration (FIC) for each fixed ratio of each combination are summarized in Table 2. The sum FIC values obtained were then used to construct isobolograms (Figure 1).

Interaction of lithium chloride with RWJ67657

The mean sums of FIC values of the interaction of lithium chloride with RWJ67657 are shown in Table 2. The isobologram for the interaction of lithium chloride with RWJ67657 (Figure 1A) tended towards antagonism at all ratios tested except for ratio 20:80 and 10:90 with mean range sums of FIC of 1.841 (0.839-2.425).

Interaction of SB216763 and BIO with RWJ67657

The isobolograms for the interactions between SB216763 and BIO with RWJ67657 are presented in Figures 1B and 1C, and the mean sums of FIC values are presented in Table 2. The interactions against 3D7 strain from both combinations were synergistic. The mean range sums of FIC values of SB216763 and BIO with RWJ67657 against 3D7 cultures obtained were 0.876 (0.732-0.929) and 0.798 (0.554-0.953) respectively.

DISCUSSION

The current study is an investigation on the individual *ex vivo* antiplasmodial activities of a panel of GSK3 inhibitors (LiCl, kenpaullone, BIO and SB216763); and two p38 MAPK inhibitors (RWJ67657 and SB202190) against chloroquine-sensitive 3D7 strain of *P. falciparum*. Three of these GSK3 inhibitors, LiCl, BIO and SB216763 were then selected based on variations in their GSK3-inhibitory mechanisms for combination experiments against 3D7 with the more potent antiplasmodial p38 MAPK inhibitor, RWJ67657. The present study is an effort to elucidate the role of GSK3 and MAPK signaling during malarial parasite development.

Both GSK3 and p38 MAPK inhibitors tested individually showed potent inhibitory activities on replication of *P. falciparum* 3D7 cultured *ex vivo* in erythrocytes. This concur with previous reports on the effects of kinase inhibitors on *P. falciparum* i.e. inhibition of recombinant *P. falciparum* GSK3 by GSK3 inhibitors (Drocheau *et al.,* 2004) and antiplasmodial activities of MAPK inhibitors against *P. falciparum* in culture (Brumlik *et al.,* 2011). We have also previously demonstrated chemo-suppressive effects of LiCl on *P. berghei* development in a murine model of malarial infection (Nurul Aiezzah *et al.,* 2010).

Among apicomplexan parasites, the essential roles of GSK3 and MAPK in parasite development as implicated from inhibitor studies described above, are not limited to the plasmodial sp. In human African trypanosomiasis, a short protein isoform of *Trypanosoma brucei* GSK3 was shown to be essential for parasite growth and viability (Ojo *et al.,* 2008). Also Xingi *et al.* (2009) provided evidence that *Leishmania donovani* GSK3 was involved in cell-cycle control and pathways leading to apoptosis-like death. Additionally, Wei *et al.* (2002) and Pfister *et al.* (2006) reported direct involvement of p38 MAPK in the developmental stages of *Toxoplasma gondii* and *Trypanosoma brucei* respectively.

Noteworthy, findings from the present study revealed that ATP-competitive and more specific pharmacological inhibitors of GSK3 (kenpaullone, BIO and SB216763) were more potent at suppressing growth of *P. falciparum* 3D7 compared to the non-ATP-competitive GSK3 inhibitor, LiCl. All GSK3 and MAPK inhibitors tested, however arrested the

trophozoite stage of *P. falciparum* growth thus possibly implicating similar targets for both kinases in parasite development.

When tested in combination with the MAPK inhibitor, RWJ67657 against the 3D7 parasite strain, BIO and SB216763 each displayed synergistic interactions with more profound inhibitory effects on parasite growth. In contrast, LiCl-RWJ67657 combination resulted in an antagonistic relationship. In other words, LiCl exhibited less favorable inhibition with a higher IC_{50} on parasite growth upon combination with the p38 MAPK inhibitor.

As BIO and SB216763 are two ATP-competitive inhibitors which both negatively affect phosphorylation of Tyr 216 in GSK3 (Meijer *et al.,* 2003; Schutz *et al.,* 2011), it is tempting to suggest that the enhanced antiplasmodial actions seen here with these inhibitors when combined with RWJ67657 may be attributed in part to their effects on the tyrosine phosphorylation. The results shown here is in agreement with Drocheau *et al.* (2004) who reported that residues thought to be involved in the activation of GSK3 through tyrosine phosphorylation are conserved in *P. falciparum.* LiCl, in contrast is a non-ATP-competitive inhibitor in which inhibition of GSK3 is by direct competition with magnesium ions; or indirectly via enhanced serine phosphorylation and autoregulation (Klein & Melton 1996; Kirshennboim *et al.,* 2004).

Upstream signaling pathways mediated by MAPKs reported to eventually lead to GSK3 inhibition include a pathway which involves phosphorylation of a serine residue by a MAPK-activated protein kinase (Cohen & Frame, 2001). In addition, Ding *et al.* (2005) reported that active ERK2 phosphorylated a Thr 43 residue in human GSK3β. p38 MAPK has also been reported to specifically phosphorylate GSK3â at residue Thr 390 (Thornton *et al.,* 2008).

Based on our findings described here, both combinations of SB216763 and BIO with RWJ67657 showed greater inhibition compared to their individual effects implying that GSK3 and MAPK have coordinated actions. This synergistic interaction suggests that combined inhibition of GSK3 and MAPK is a plausible approach for development of a more potent antimalarial drug.

ACKNOWLEDGEMENTS

This work was supported by funding from a Fundamental Research Grant Scheme (FRGS/1/2012/ST04/UKM/02/4) and a Research University Grant (UKM-GUP-2011-212).

REFERENCES

Berenbaum, M.C. 1978. A method for testing for synergy with any number of agents. *The Journal of Infectious Disease,* **137**: 122-130.

Brumlik, M.J., Nkhoma, S., Kious, M.J., Thompson III, G.R., Patterson, T.F., Siekerka, J.J., Anderson, T.J.C. & Curiel, T.J. 2011. Human p38 mitogen-activated protein kinase inhibitor drugs *Plasmodium falciparum* replication. *Experimental Parasitology,* **128**: 170-175.

Canfield, C.J., Pudney, M. & Gutteridge W.E. 1995. Interactions of atovaquone with other antimalarial drugs against *Plasmodium falciparum in vitro*. *Experimental Parasitology*, **80**: 373-381.

Chen, Z., Gibson, T.B., Robinson, F., Silvestro, L., Pearson, G., Xu, B.-E., Wright, A., Vanderbilt, C. & Cobb, M.H. 2001. MAP kinases. *Chemical Reviews,* **101**: 2449-2476.

Cohen, P. 2002. Protein kinases – the major drug targets of the twenty-first century? *Nature Reviews Drug Discovery* **1**: 309-315.

Cohen, P. & Frame, S. 2001. The renaissance of GSK3. *Nature Reviews Molecular Cell Biology*. **2**: 769-776.

Ding, Q., Xia, W., Liu, J.C., Yang, J.Y., Lee, D.F., Xia, J., Bartholomeusz, G., Li, Y., Pan, Y., Li, Z., Bargou, R.C., Qin, J., Lai, C.C., Tsai, F.J., Tsai, C.H. & Hung, M.C. 2005. Erk associates with and primes GSK-3β for its inactivation resulting in up-regulation of β-catenin. *Molecular Cell.* **19**: 159-170.

Doble, B.W. & Woodgett, J.R. 2003. GSK-3: tricks of the trade for a multitasking kinase. *Journal of Cell Science*, **116**: 1175-1186.

Dobson, S., Bracchi, V., Chakrabarti, D. & Barik, S. 2001. Characterization of a novel serine/threonine protein phosphatase (PfPPJ) from the malaria parasite, *Plasmodium falciparum*. *Molecular and Biochemical Parasitolology*, **115**: 29-39.

Doerig, C. 2004. Protein kinases as targets for anti-parasitic chemotherapy. *Biochimica et Biophysica Acta,* **1697**: 155-168.

Drocheau, 2004. *Plasmodium falciparum* Glycogen Synthase Kinase-3: Molecular Model, Expression, Intracellular Localisation and Selective Inhibitors, *Biochimica et Biophysica Acta.* **1697**: 181-196.

Embi, N., Rylatt, D.B. & Cohen, P. 1980. Glycogen synthase kinase-3 from rabbit skeletal muscle; separation from cyclic-AMP-dependent protein kinase and phosphorylase kinase. *European Journal of Biochemistry.* **107**: 519-527.

Hommes, D.W., Peppelenbosch, M.P. & Van Deventer, S.J.H. 2003. Mitogen activated protein (MAPK) kinase signal transduction pathways and novel anti-inflammatory targets. *GUT*. **52**: 144-151.

Gupta, S., Thapar, M.M., Wernsdorfer, W.H. & Bjorkman, A. 2002. *In vitro* interactions of artemisinin with atovaquone, quinine, and mefloquine against *Plasmodium falciparum. Antimicrobial Agents and Chemotherapy,* **46**: 1510-1515.

Kebeneil, J.S., Ndalut, P.K. & Sabah, A.O. 2004. Synergism of artemisinin with abyssinone – V from *Erythrina abyssinica* (Lam. ex) against *Plasmodium falciparum* parasites: A potential anti-malarial combination therapy. *Journal of Medicinal Plants Research.* **5** :1355-1360.

Klein, P.A & Melton, D.A. 1996. A molecular mechanism for the effect of lithium on development. *Proceedings of the National Academy of Sciences,* **93**: 8455-8459.

Kim, E.K. & Choi, E.J. 2010. Pathological roles of MAPK signaling pathways in human disease. *Biochimica et Biophysica Acta.* **1802**: 396-405.

Kirshennboim, N., Plotkin, B., Ben, Shlomo, S., Kaidanovich-Beilin, O. & Eldar-Finkelman, H. 2004. Lithium-mediated phosphorylation of glycogen synthase kinase-3 involves PI3Kinase-dependent activation of protein kinaseC- alpha. *Journal of Molecular Neuroscience.* **24**: 199-207.

Lawrence, M.C., Jivan, A., Shao, C., Duan, L., Goad, D., Zaganjor, E., Osborne, J., McGlynn, K., Stippec, S., Earnest, S., Chen, W. & Cobb, M.H. 2008. Review: The roles of MAPKs in disease. *Cell Research*. **18**: 436-442.

Makler, M.T., Ries, J.M., Williams, J.A., Bancroft, J.E., Piper, R.C., Gibbons, B.L. & Hinrichs, D.J. 1993. Parasite lactate dehydrogenase as an assay for *Plasmodium falciparum* drug sensitivity. *The American Journal of Tropical Medicine and Hygiene,* **48**: 739-741.

Meijer, L., Skaltsounis, A.L., Magiatis, P., Polychronopoulos, P., Knockaert, M., Leost, M., Ryan, X.P., Vonica, C.A., Brivanlou, A., Dajani, R., Crovace, C., Tarricone, C., Musacchio, A., Roe, S.M., Pearl, L. & Greengard, P. 2003. GSK-3-Selective Inhibitors Derived from Tyrian Purple Indirubins. *Cell press*. **10**: 1255-1266.

Meijer, L., Flajolat, M. & Greengard, P. 2004. Pharmacological inhibitors of glycogen synthase kinase 3. *Trends in Pharmacological Sciences,* **25**(9): 471-480.

Nurul Aiezzah, Z., Noor, E. & Hasidah, M.S. 2010. Suppression of *Plasmodium berghei* parasitemia by LiCl in an animal infection model. *Tropical Biomedicine*. **27**(3): 624-631.

Ojo, K.K., Gillespie, J.R., Riechers, A.J., Napuli, A.J. & Verlinde, C.L. 2008. Glycogen synthase kinase 3 is a potential drug target for African trypanosomiasis therapy. *Antimicrobial Agents Chemotherapy*. **52**: 3710-3717.

Pfister, D.D., Burkard, G., Morand, S., Renggli, C.K., Roditi, I. & Vassella, E. 2006. A Mitogen-Activated Protein Kinase Controls Differentiation of Bloodstream Forms of *Trypanosoma brucei. Eukaryotic Cell*, **5**: 1126-1135.

Radfar, A., Mendez, D., Moneriz, C., Linares, M., Marin-Garcia, P., Puyet, A., Diez, A. & Bautista, J.M. 2009. Synchronous culture of *Plasmodium falciparum* at high parasitemia levels. *Nature Protocols*, **4**: 1899-1915.

Schutz, S.V., Schrader, A.J., Zengerling, F., Genze, F., Cronauer, M.V. & Schrader, M. 2011. Inhibition of Glycogen Synthase Kinase-3β Counteracts Ligand-Independent Activity of the Androgen Receptor in Castration Resistant Prostate Cancer. *PLOS ONE*, **6**: e25341.

Skinner, T.S., Manning, L.S, Johnston, W.A. & Davis, T.M.E. 1996. *In vitro* Stage-specific Sensitivity of *Plasmodium falciparum* to Quinine and Artemisinin Drugs. *International Journal for Parasitology*, **26**: 519-525.

Thornton, T.M., Pedraza-Alva, G., Deng, B., Wood, C.D., Aronshtam, A., Clements, J.L., Sabio, G., Davis, R.J., Matthews, D.E., Doble, B. & Rincon, M. 2008. Phosphorylation by p38 MAPK as an alternative pathway for GSK3beta inactivation. *Science,* **320**(5876): 667-670.

Winstanley, P.A. 2000. Chemotherapy for *P. falciparum* malaria: The Armoury, The Problems And The Prospects. *Parasitology Today,* **16**: 146-153.

Wei, S., Marchesb, F., Daniela, B., Sondac, S., Heidenreichd, K. & Curiela, T. 2002. Pyridinylimidazole p38 mitogen-activated protein kinase inhibitors block intracellular *Toxoplasma gondii* replication. *International Journal for Parasitology,* **32**: 969-977.

World Health Organization (WHO). 2012. World malaria report 2012. 195 pp

Xingi, E., Smirlis, D. & Myrianthopoulos, V. 2009. 6-Br-5-methylindirubin-3 'oxime (5-Me-6-BIO) targeting the leishmanial glycogen synthase kinase-3 (GSK-3) short form affects cell-cycle progression and induces apoptosis-like death: Exploitation of GSK-3 for treating leishmaniasis. *International Journal for Parasitology*, **39**: 1289-303.